

Hyphal activity in mull and mor of an oak forest

INTRODUCTION

In the literature mor has been stated to have a high mycelium content, mull a low content. This difference in growth of fungi has even been considered a characteristic distinguishing feature between mull and mor (Müller 1887, Waksman 1932, Wilde 1954). Though Müller has found a rich growth of fungi in mull, the hyphae were very thin while the hyphae in mor were much thicker and more conspicuous. Meyer (1959), however, has found that the numbers of fungi in mull and mor do not show much difference when the numbers of fungi found by the dilution plate method are expressed per gram of organic matter. Witkamp (1960), using the dilution plate method, gave the number of fungi per gram of dry soil and found a greater number of fungi in mor than in mull. According to him, also the mycelium content and the growth rate in the soil are greater in the mor that he studied than in mull. Jensen (1963) also used the dilution plate method for counting fungi in various mull and mor soils. He stated that per gram of dry soil, mor contains 3-4 times as many fungi as does mull, but that per gram of organic matter the number of fungi is slightly higher in mull than it is in mor.

However, the dilution plate method is not a very satisfactory technique for studying fungi in soil (a.o. Waksman 1932, Gams and Parkinson 1960, Warcup 1960), with which Jensen (1963) agrees. Direct observation (method Jones and Mollison 1948) also does not provide wide data on the real activity of the fungi, which consists in the growth rate of their mycelium. It is, therefore, desirable to compare fungi in mull and mor in a somewhat different way.

¹⁾ Institute for Biological Field Research, Arnhem, Netherlands

Sampling area and methods

We made our observations from August 1963 till December 1965 in the same area as Witkamp (1960). It was described by Minderman (1960). Only the fungus-counts by means of the dilution plate method were carried out from March 1962 till July 1963. The dilution plate counts were made on cherry-agar. The mycelium content of soil was determined monthly by the method of Jones and Mollison (1948). However, we preferred chlorazol black as a dye, a chitin-stain which selectively stains the hyaline hyphae. Growth of mycelium was determined on nylon-gauze which was left in the soil for three weeks. This method by Waid and Woodman (1957) is subject to the difficulty that in the field the circumstances (temperature and moisture) are not constant, and therefore the period during which optimal growth over the gauze takes place varies in length. However, this objection also applies to other methods for determining growth rate. The nylon-gauze method has the advantage that there is less condensation of water than occurs on Rossi-Cholodny slides, and that three-dimensional growth is possible. The gauze we used was Monodur 40 (125 meshes per cm). For each monthly count we used 3 pieces of gauze in which we counted in 600 meshes.

The number of mycolytic organisms was counted by the method of Carter and Lockwood (1957a).

For the determination of species of mycelium present in soil or gauze a washing apparatus as described by Parkinson and Williams (1961) was used. The washed soil or bits of nylon-gauze were put on the surface of cherry-agar or potato-dextrose-agar plates.

DILUTION PLATE COUNTS

Although we set little value upon data from plate counts we summarize them in a Table (1) in order to facilitate comparison of our further results with data from the literature.

In this case there is only a difference in counts per gram of dry soil between those in mull rich in lime, and those in mor and acid mull. Yet our results lead to the same conclusions as made by Jensen (1963), viz., that the difference between mull and mor does not show in numbers of fungi per gram of organic matter.

Table 1: Average of 10 counts in the period March 1962-July 1963

depth of mineral soil	Ca-mull	acid mull	mor	Ca-mull	acid mull	mor
0 cm	867	3,513	5,256	7,680	13,710	9,450
5 cm	194	981	796	3,441	14,680	9,629
20 cm	23	197	96	1,450	4,287	3,462
	Fungal counts in thousands per g of dry soil			Fungal counts in thousands per g of organic matter		

MYCELIUM CONTENT AND HYPHAL GROWTH

The mycelium content of the 5 and 10 cm layers shows a density similar to that of the 0 cm layer (Fig. 1) of each forest floor type, but at a somewhat lower level. The data obtained from the measurements correspond with data in the literature on a high content of mycelium in mor, and a low content in mull. It is, however, not possible to distinguish between active and dead mycelium. Determining the growth rate is, until now, the only way to find out about activity of fungi. The known methods for determining growth rate have their shortcomings. None will give a true picture of the growth in the soil that really occurs.

Table 2: Average of monthly estimations in the period August 1963-December 1965

depth of mineral soil	Ca-mull	acid mull	mor	Ca-mull	acid mull	mor	Ca-mull	acid mull	mor
0 cm	178	258	924	1,778	1,835	4,606	15,4	14,0	13,9
5 cm	128	131	346	2,178	1,969	6,182	8,7	7,4	13,0
10 cm	98	108	207	2,874	2,284	8,112	7,6	5,1	10,7
	Mycelium content in meters per gram of dry soil			Mycelium content in meters per gram of organic matter			Mycelium growth expressed as percentage of meshes of nylon-gauze with hyphae		

No difference between mull and mor as to growth of mycelium has become apparent in the 0-cm layer (Fig. 2). Also the 5 and 10 cm layers do not show considerable differences between mycelium growth in mull and mor.

The data of Table 2 show that differences between the decomposition types as to mycelium content of the soil have to be ascribed to a difference in breakdown rates of mycelium and not to a difference in activity of the fungi in mull and mor.

BREAKDOWN OF MYCELIUM IN SOIL

Little is known about breakdown of mycelium in soil. Bacteria and actinomycetes are considered the principal cause (Carter and Lockwood 1957 a, b, Mitchell and Alexander 1963). In addition it is possible that mycelium is broken down chemically and enzymatically (Tracey 1956, Carter and Lockwood 1957b). In the mull and mor that we studied breakdown probably takes place in various ways, as suggested by the accumulation of mycelium in mor. The number of bacteria in mull rich in lime is much higher than in mor (Witkamp, 1960). Also in the composition of bacterial populations as to species mull differs greatly from mor (J. C. Went, unpublished). Mycolytic microorganisms are not found in mor. In mull they occur especially in spring (Table 3). So far isolation of the bacteria and actinomycetes was not quite successful.

Table 3: Number of mycolytic micro-organisms per cm of mycelium in the soil.

	March ' 63	April ' 63	May ' 63
Ca - mull			
mineral soil 0 cm	143	10	52
5 cm	87	24	2
acid mull			
mineral soil 0 cm	1	1	0
5 cm	0	0	0

The speed with which mycelium is broken down can be derived from the mycelium content of soil and its growth rate. The percentage growth over nylon gauze provides a measure for the growth rate. For the mycelium content we use the figures per gram of dry soil. These figures can more easily be related to a constant volume of soil than those per gram of organic matter. The quotient of growth and quantity of mycelium is a measure for the relative breakdown rate. If the breakdown in mull

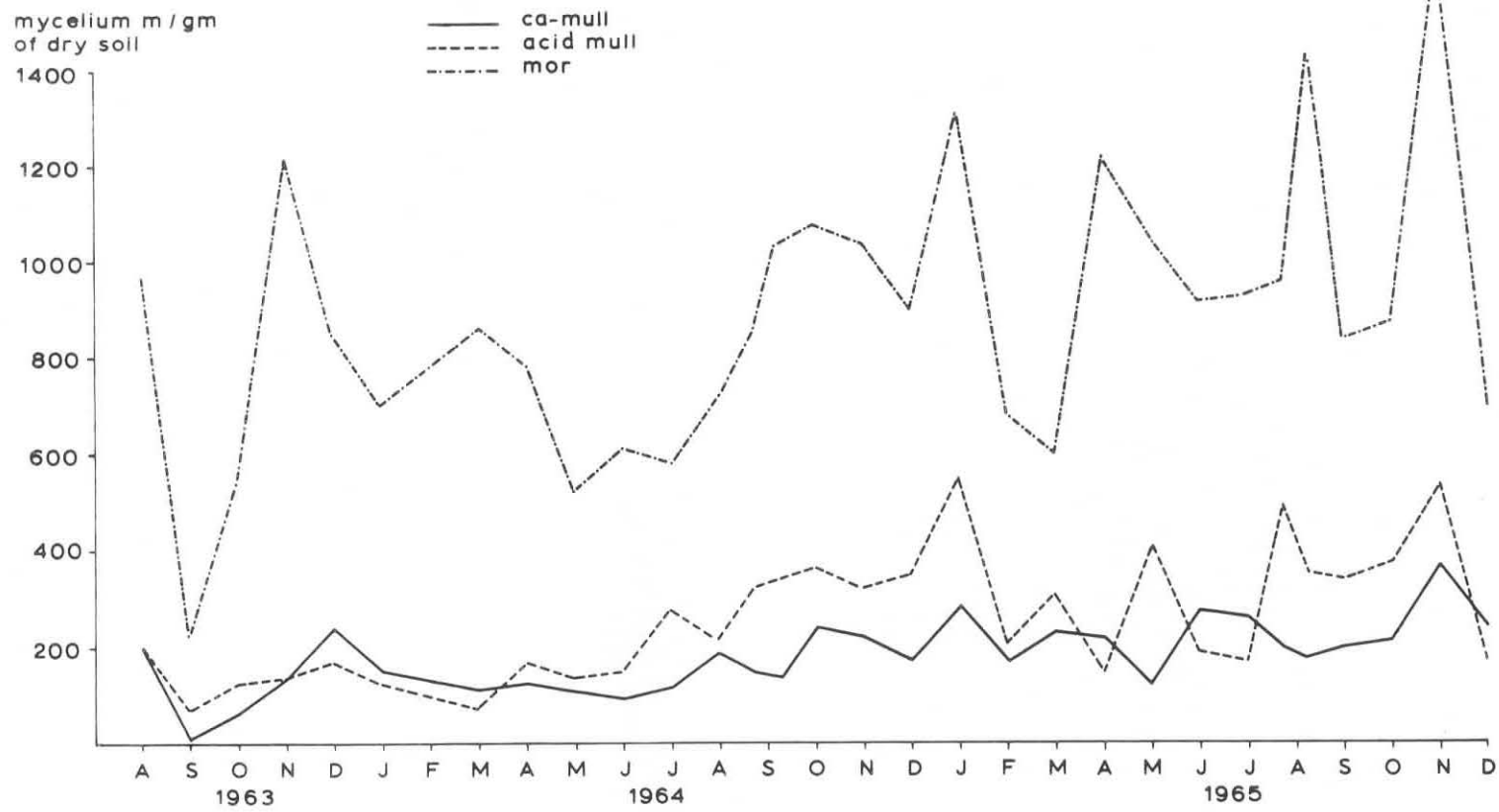


Fig. 1
Mycelium content of 0-cm layer of the mineral soil.

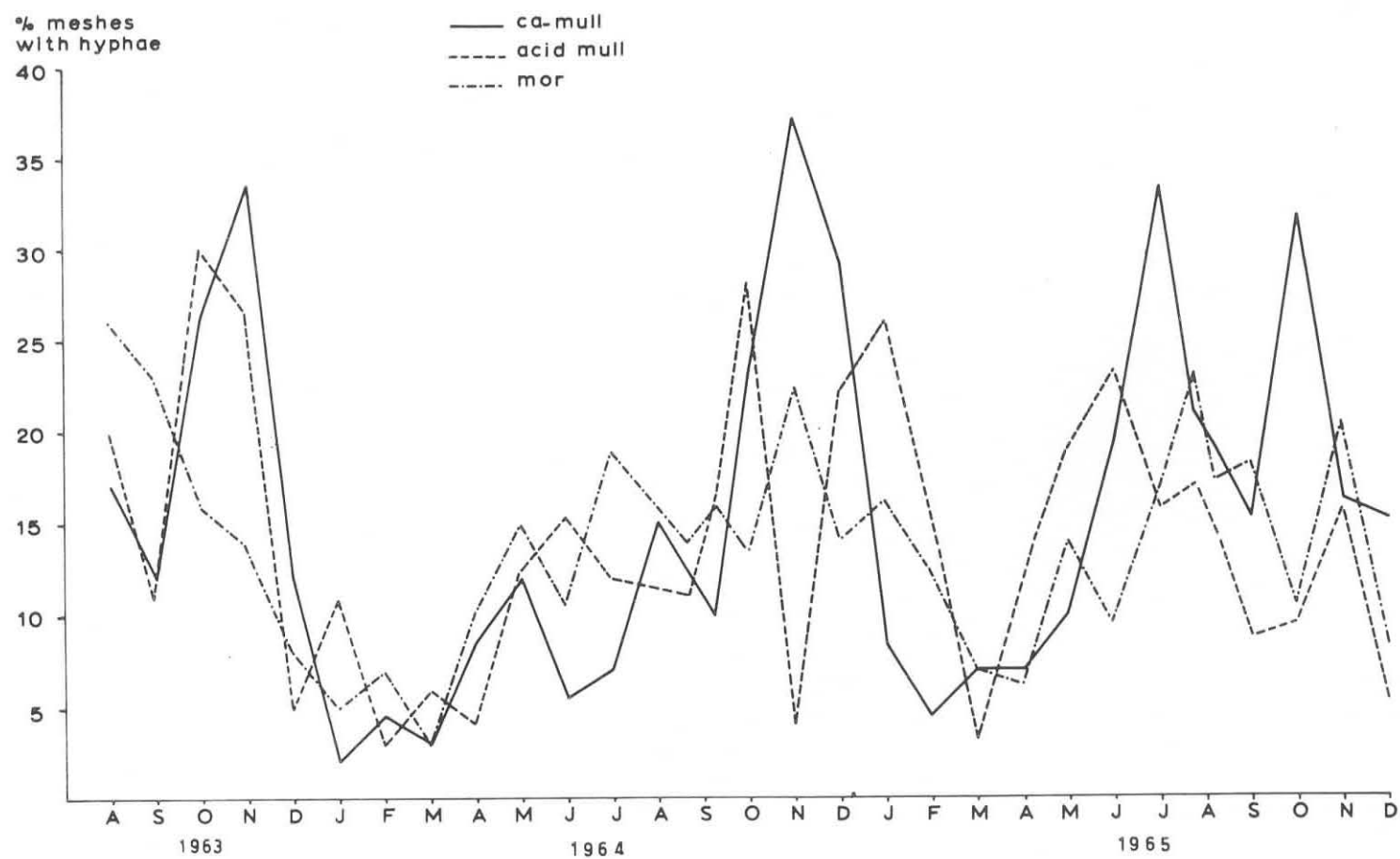


Fig. 2

Percentage meshes of nylon-gauze with new grown mycelium after three weeks exposure in 0-cm layer of the mineral soil.

rich in lime during the total period of observation is placed at 1, then the breakdown rate in some other type of forest floor or another layer (x) can be calculated from the values in Table 2 as follows:

$$\frac{\% \text{ growth x}}{\text{mycelium content x}} \times \frac{\text{mycelium content Ca-mull 0-cm}}{\% \text{ growth Ca-mull 0-cm}} \times 1.0$$

From Figs. 1 and 2 one can deduce that as to fungi the year can be divided into three periods. The relative breakdown-rate has been calculated for the whole period, as well as for these seasons (Table 4).

Table 4: Relative breakdown rate of mycelium

depth of mineral soil	average breakdown rate during whole period	autumn ' 63	winter ' 63/' 64	spring/ summer ' 64	autumn ' 64	winter ' 64/' 65	spring/ summer ' 65	autumn ' 65
Ca-mull								
0 cm	1.0	2.6	0.3	0.9	1.2	0.7	0.9	1.1
5 cm	0.8	2.2	0.4	1.4	0.7	0.4	0.7	0.8
10 cm	0.9	-	-	0.6	0.8	0.6	0.7	1.5
acid mull								
0 cm	0.6	1.9	0.6	0.7	0.5	0.5	0.9	0.4
5 cm	0.7	1.8	0.8	1.0	0.4	0.6	0.5	0.4
10 cm	0.6	-	-	1.8	0.5	0.7	1.2	0.4
mor								
0 cm	0.2	0.5	0.1	0.3	0.2	0.2	0.1	0.2
5 cm	0.4	1.8	0.2	0.3	0.3	0.7	0.3	0.4
10 cm	0.6	-	-	0.5	0.6	0.7	0.4	1.2

The numbers of Table 4 we cannot interpret until we know more about the nature of the processes that play a rôle in the breakdown of mycelium. The only conclusions that we could draw at present are:

- The breakdown of mycelium in the two types of mull occurs to a depth of 10 cm with the same speed.
- With increasing depth breakdown of mycelium in mor is accelerated.
- Breakdown in both types of mull is distinctly greater than it is in mor.
- Seasonal differences in breakdown rate are not distinct. It would seem, however, that in the mull rich in lime the maximum of breakdown lies in the autumn, but in acid mull in spring/summer.

NATURE OF THE MYCELIUM

Various authors have found that a considerable part of the mycelium in mor consists of thick brown hyphae (Müller 1887, Meyer 1959, Witkamp 1960). Sometimes they add that this is mycelium of Basidiomycetes. In mor we do find more mycelium than in mull. However, it is a matter for consideration whether this browning is not rather a result of age and environment. Waid and Woodman found that nylon-gauze after long exposure in the soil contains more brown hyphae than gauze that has been in the soil for only a short time (Waid 1960). Young mycelium growing over nylon is always transparent, except when it has grown through a humic mass such as sometimes is deposited in the meshes of the gauze. There are no indications that this brown mycelium belongs to Basidiomycetes only.

The washing technique of Parkinson and Williams (1961) can also be used for nylon-gauze that for some time has been in the soil. In this way one can determine which are the actively growing species of fungi in the soil at a certain moment. As yet not enough work has been done in this field. We therefore mention only a few results. Characteristic is a washing of nylon-gauze from which from the mull rich in lime 32 species, from the acid mull 24 species and from mor 14 different species were isolated. In mor, washed soil as well as washed nylon-gauze shows a dominance of *Trichoderma viride* ($\pm 40\%$) besides *Mortierella rammaniana* ($\pm 30\%$). These species also prevail in the acid mull but less strongly. In mull rich in lime no single species is dominant. The results from washed nylon-gauze agree reasonably well with those from washed soil. However, up till now both methods of making an inventory have not been carried out simultaneously.

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DISCUSSION

K. HAIDER: Is there any method to estimate the number of fungi in soil and what method would you propose?

Mrs. NAGEL-DE BOOIS: The Jones and Mollison slide method will give the total length of mycelium in soil. Vital staining of this mycelium and estimation of mycelial growth activity will complete the information.

O. W. HEAL: In relation to the previous answer do you think that it is possible to distinguish approximately between live and dead hyphae by staining the Jones and Mollison films?

Mrs. Nagel has made a very important point that the amount of mycelium in a soil may reflect only the rate of decomposition of the mycelium. Miss Latter at Merlewood has shown that, in four soils at Moor House, the percentage of hyphae which are retained (alive) varies from 56 % in a well drained mineral grassland to 7 % in a non-vegetated, waterlogged peat. This is positively correlated with the rate of colonisation of slide traps, the rate of cellulose decomposition and the respiration rate of the four soils. This apparently confirms Mrs. Nagel's point that estimates of total length of mycelium are not the best estimates of fungal activity.

Mrs. NAGEL-DE BOOIS: Until now we did not stain the films. But another question is that living mycelium does not need to be the same as active mycelium. In that case we need some more information about growth-activity.

W. GAMS: Vital staining with fluorescence microscopy for the distinction between living and dead mycelium on nylon mesh was used at Merlewood (Grange-over-Sands). Although this technique is of great theoretical interest it is laborious and gives only a view of the balance between the steadily occurring new colonization and decomposition of mycelium.

I wonder whether you were really able to remove all fungal spores by washing without removing mycelium from the smooth nylon surface. When I worked with polyethylene strips, I found that this was the greatest difficulty.

Mrs. NAGEL-DE BOOIS: I'm sure I did remove some mycelium from the nylon-mesh when washing. But I can only hope that it was not washed out selectively to some species.